FIG. 1

```
                        10           20           30           40           50
                        |            |            |            |            |
Bet v 1 sense       5'- A A T T A T G A G A C T G A G A C C A C C T C T G T T A T C C C A G C A G C T C G -3'
Bet v 1 non-sense   3'- T T A A T A C T C T G A C T C T G G T G G A G A C A A T A G G G T C G T C G A G C -5'
sense primer        5'-             T G A G A C C C C T C T G T T A T C C C A G                       -3'
non-sense primer    3'-             A T A C T C T G A C T C T G G G G A G A C A                       -5'
```

FIG. 2

Oligonucleotide primers for site directed mutagenesis of Bet v 1 (No. 2801).

```
all  sense        1: 183Bv, 15-mer 5'- G T T G C C A A C G A T C A G
1    sense        2: 184Bv, 23-mer 5'- T G A G A C C C C T C T G T T A T C C C A G
1    non-sense    3: 185Bv, 23-mer 5'- A C A G A G G G G T C T C A G T C T C A T A
2    sense        4: 186Bv, 31-mer 5'- G A T A C C C T C T T C C A C A G G T G C A C C C C A A G
2    non-sense    5: 187Bv, 31-mer 5'- A C C T G T G G A A G A G G G T A T C G C C C A T C A A G G A
3    sense        6: 188Bv, 23-mer 5'- A A C A T T T C A G A A A T G G A G G G C C
3    non-sense    7: 189Bv, 23-mer 5'- T T T C C T G A A A T G T T T C A A C A C T
4    sense        8: 190Bv, 23-mer 5'- T T A A G A A C A T C A G C T T T C C C G A A
4    non-sense    9: 191Bv, 23-mer 5'- A G C T G A T G T T C T T A A T G G T T C C A
5    sense       10: 192Bv, 23-mer 5'- G G A C C A T G G C A A A C T T C A A A T A C A
5    non-sense   11: 193Bv, 23-mer 5'- A G T T T G C C A T G G T C C A C C T C C T T A
6    sense       12: 194Bv, 23-mer 5'- T T T C C C T C A G G G G C C T C C C T T C A A
6    non-sense   13: 195Bv, 23-mer 5'- A G G C C C C T G A G G G A A A G C T G A T C T T
7    sense       14: 196Bv, 24-mer 5'- T G A A G G A T C C C T C C T T G G C C T G G A A C
7    non-sense   15: 197Bv, 24-mer 5'- C C C T C C A G A T C C T T C A A T G T T T C
8    sense       16: 198Bv, 24-mer 5'- G G C A A C T G G T G A T G G A G G A T C C A T
8    non-sense   17: 199Bv, 24-mer 5'- C C A T C A C C A G T T G C C A C T A T C T T T
all  non-sense   18: 200Bv, 15-mer 5'- C A T G C C A T C C G T A A G
```
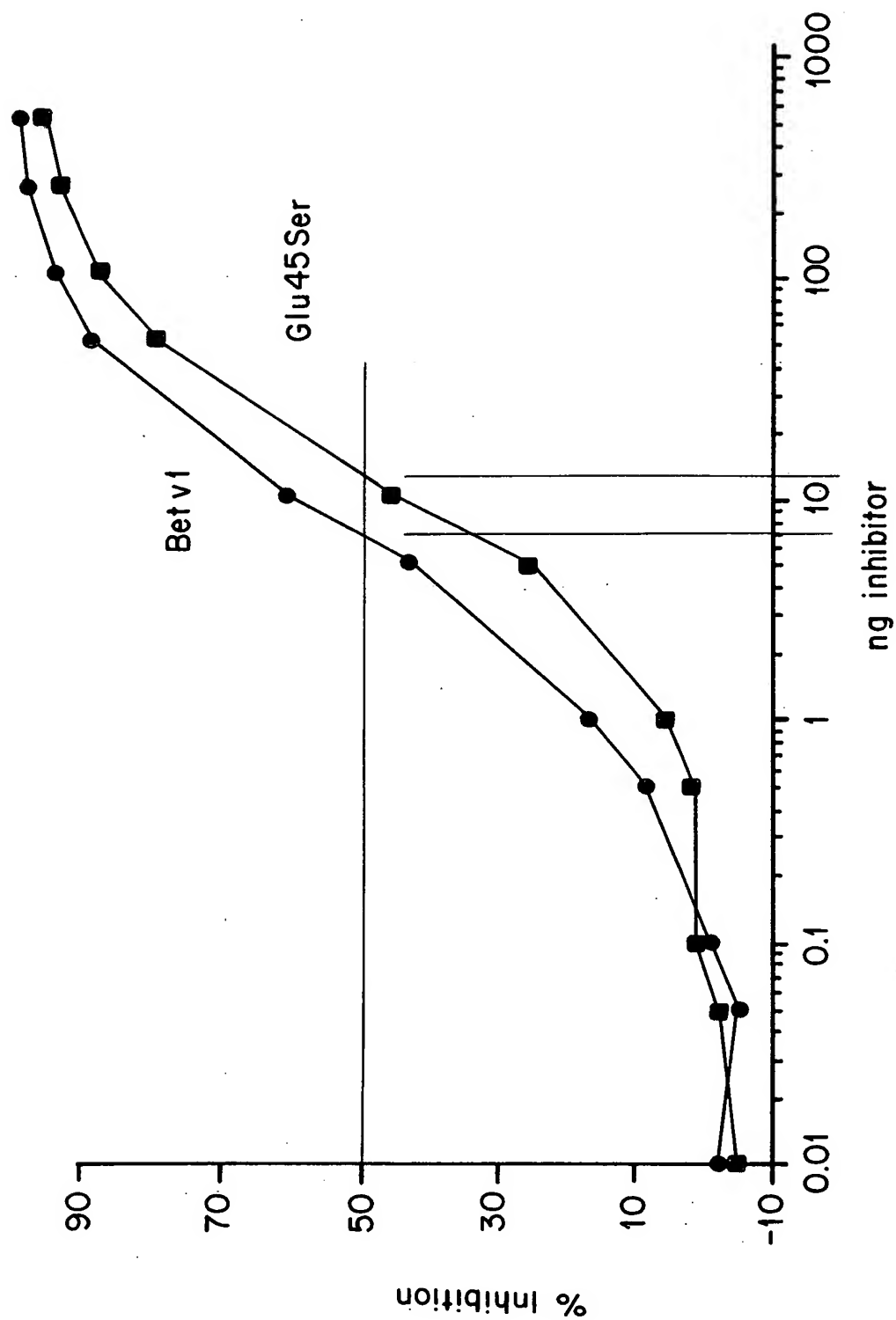
FIG. 3

1 (A- C)

GGTGTGTTTAATTATGAGACTGAGACCACCTCTGTTATCCCAGCAGCTCGACTGTTCAAG   60

 G V F N Y E   T E   T T-F S V I   P A A R L F K   20

         9 (A- G)   2 (A-C) 2 (A-C)

GCCTTTATCCTTGATGGCGATAACCTCTTTCCAAAGGTTGCACCCCAAGCCATTAGCAGT   120

  A F   I   L-D-G G   D N-T L   F   P K-Q V A   P Q   A   I   S   S   40

    3(GA- TC)   7 (AA- TC)   4 (G- C)   6 (GA- T C)

GTTGAAAACATTGAAGGAAATGGAGGGCCTGGAACCATTAAGAAGATCAGCTTTCCCGAA   180

  V E   N   I-E S G N-S G   G   P G   T   I K K-N I   S   F   P E-S   60

                                    5 (CA - TG)

GGCCTCCCTTTCAAGTACGTGAAGGACAGAGTTGATGAGGTGGACCACACAAACTTCAAA   240

  G L   P   F K   Y V   K   D   R V   D   E   V   D H-T-A N F   K   80

TACAATTACAGCGTGATCGAGGGCGGTCCCATAGGCGACACATTGGAGAAGATCTCCAAC   300

  Y   N Y   S   V   I   E   G   C   P   I G   D   T   L   E   K   I   S   N   100

10 (G AG -CAC)   8 (CCC - TGG)

GAGATAAAGATAGTGGCAACCCCCTGATGGAGGATCCATCTTGAAGATCAGCAACAAGTAC   360

  E   I   K   I   V   A   T-P-G D   G   G   S   I   L   K   I   S   N   K   Y   120

CACACCAAAGGTGACCATGAGGTGAAGGCAGAGCAGGTTAAGGCAAGTAAAGAAATGGGC   420

  H   T   K   G   D   H   E   V   K   A   E   Q   V   K   A   S   K   E   M   G   140

GAGACACTTTTGAGGGCCGTTGAGAGCTACCTCTTGGCACACTCCGATGCCTACAACTAA   480

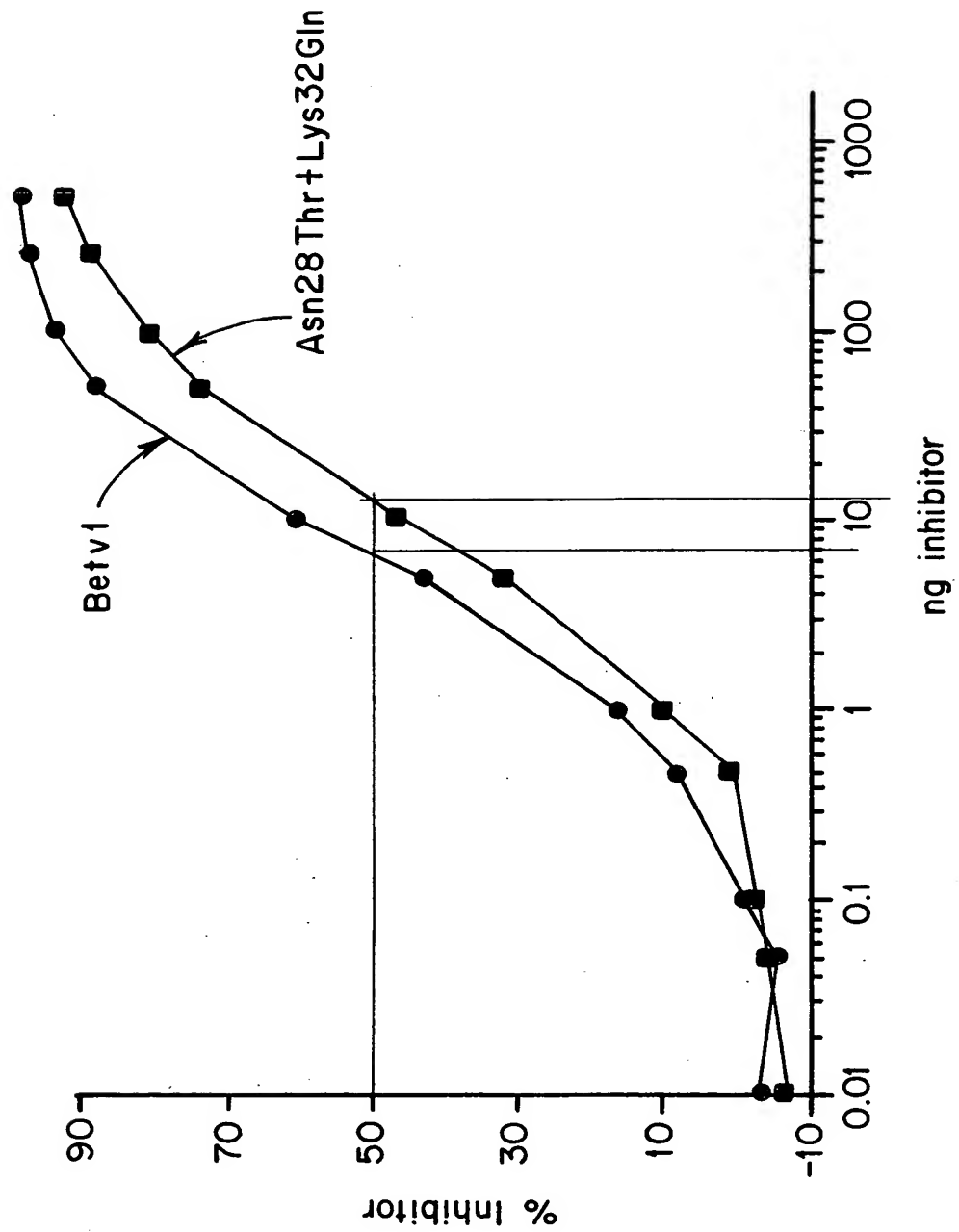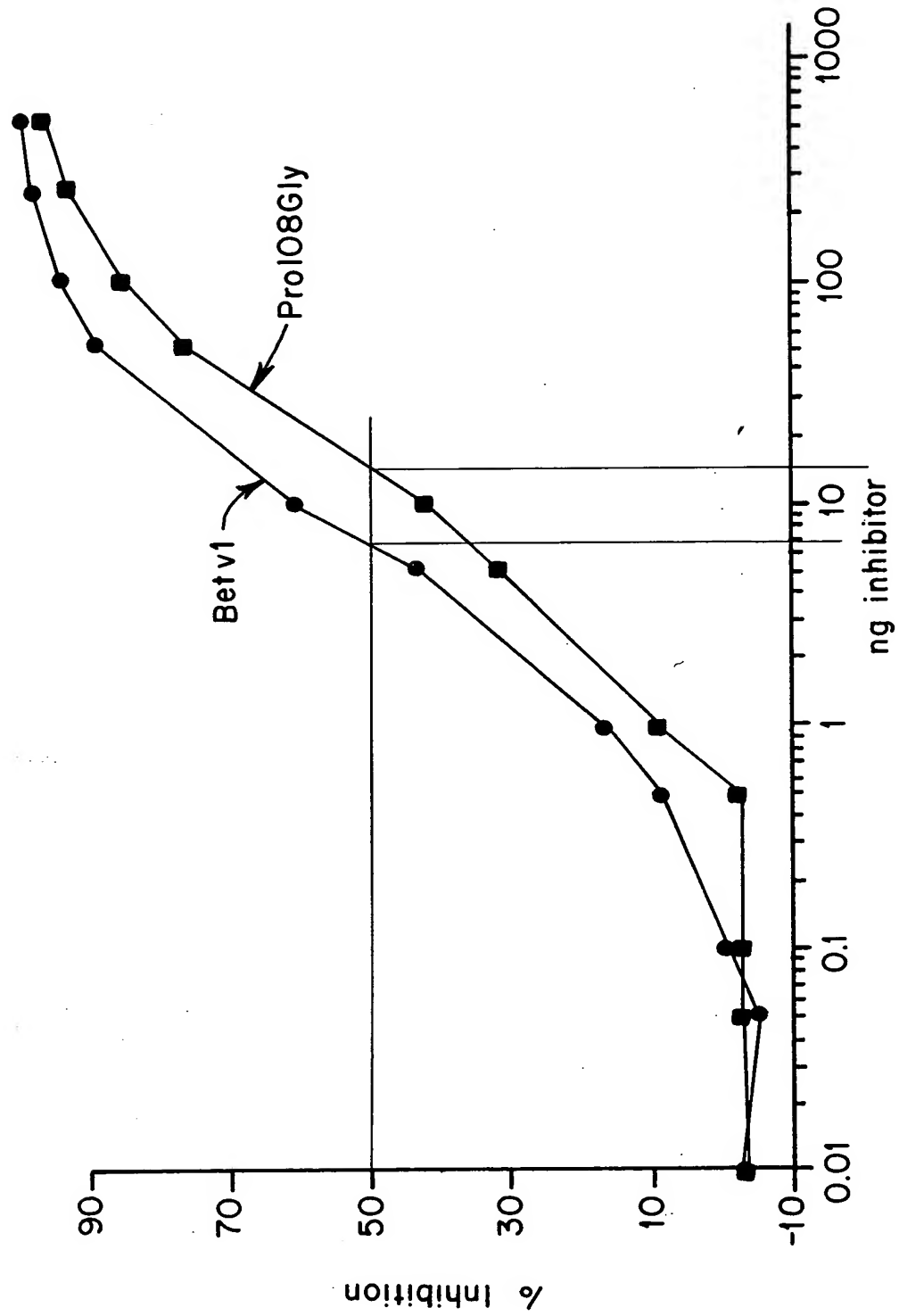 E   T   L   L   R   A   V   E   S   Y   L   L   A   H   S   D   A   Y   N stop   159
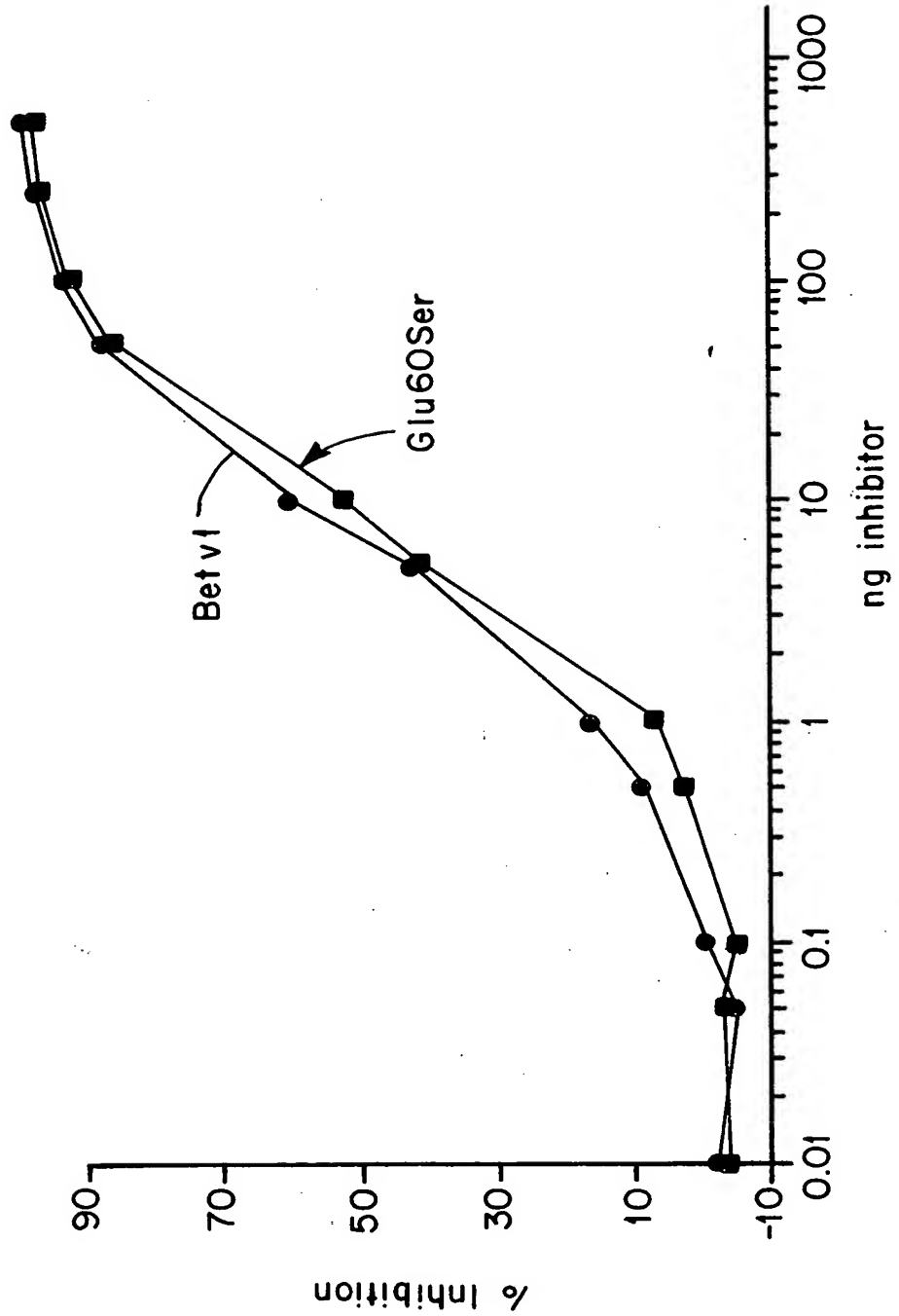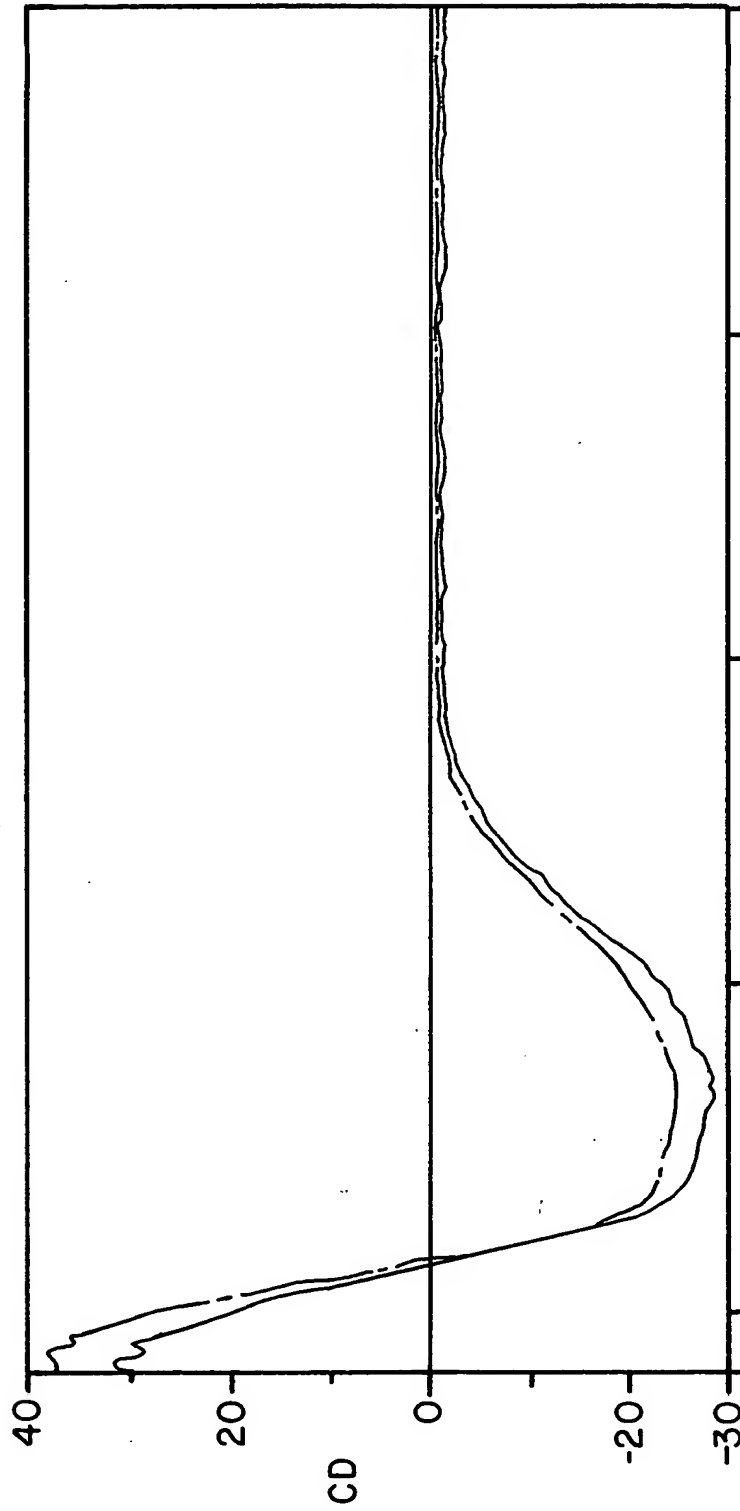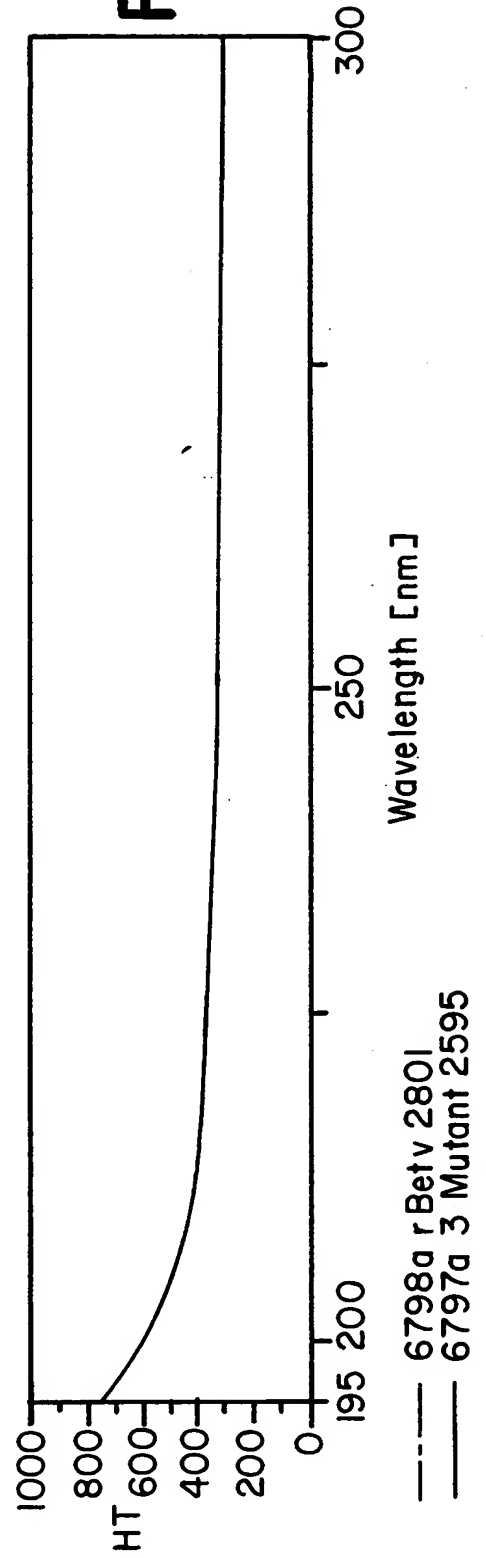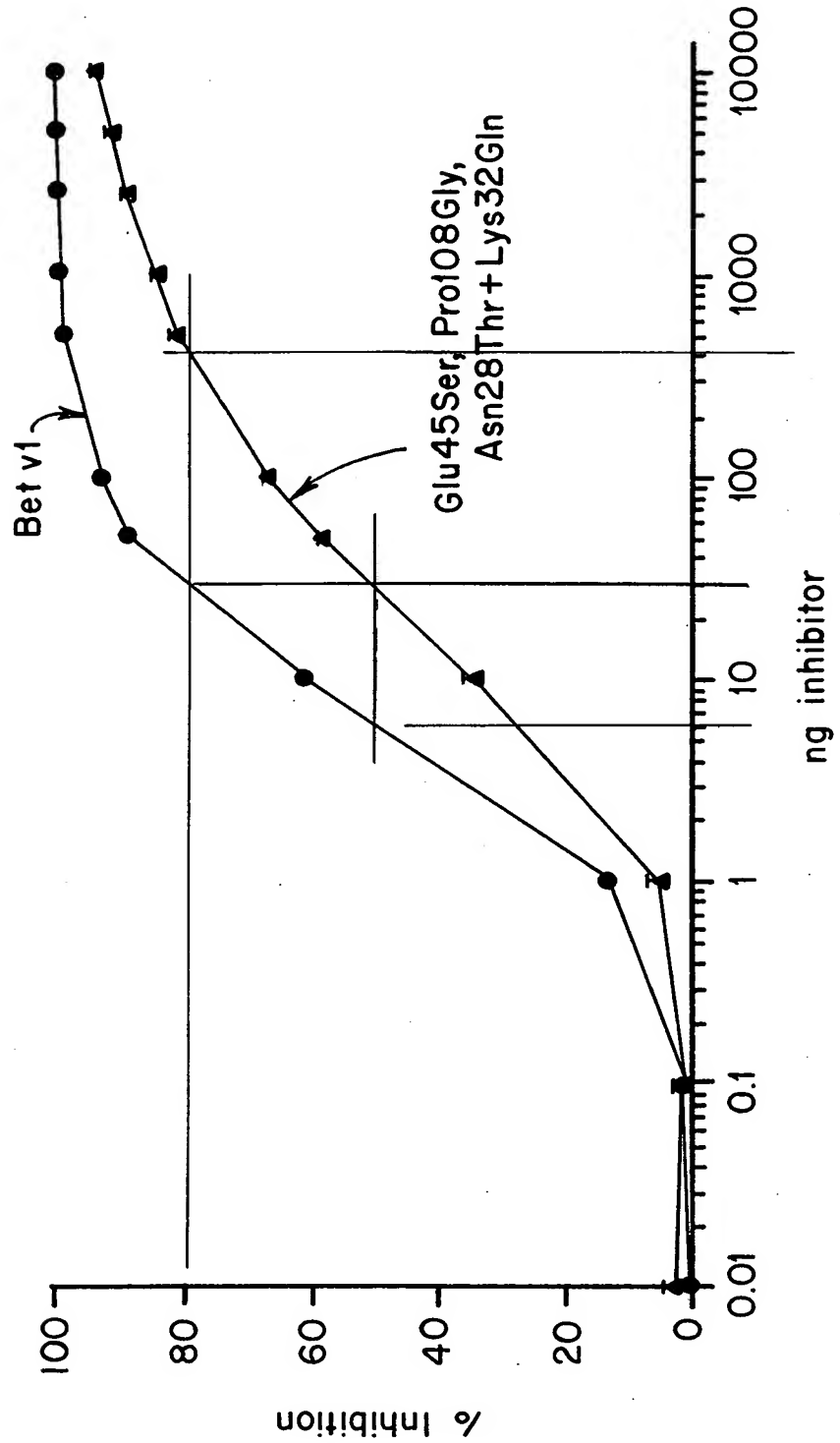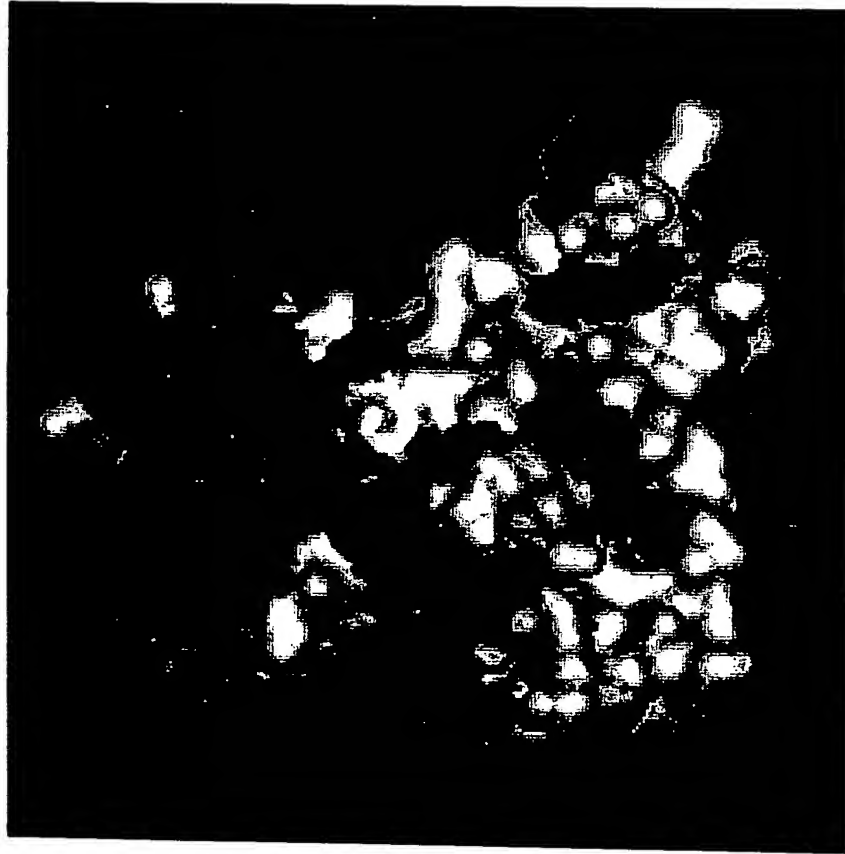
FIG. 4

% Inhibition vs ng inhibitor

Glu45Ser

Bet v1

FIG. 5

FIG. 6

FIG. 7

FIG. 8A

FIG. 8B

Wavelength [nm]

6798a r Bet v 280I
6797a 3 Mutant 2595

CD

40 20 0 -20 -30

HT

1000 800 600 400 200 0

195 200 250 300

FIG. 9

FIG. IOA

FIG. 10B

11/16

FIG. 10C

FIG. 10D
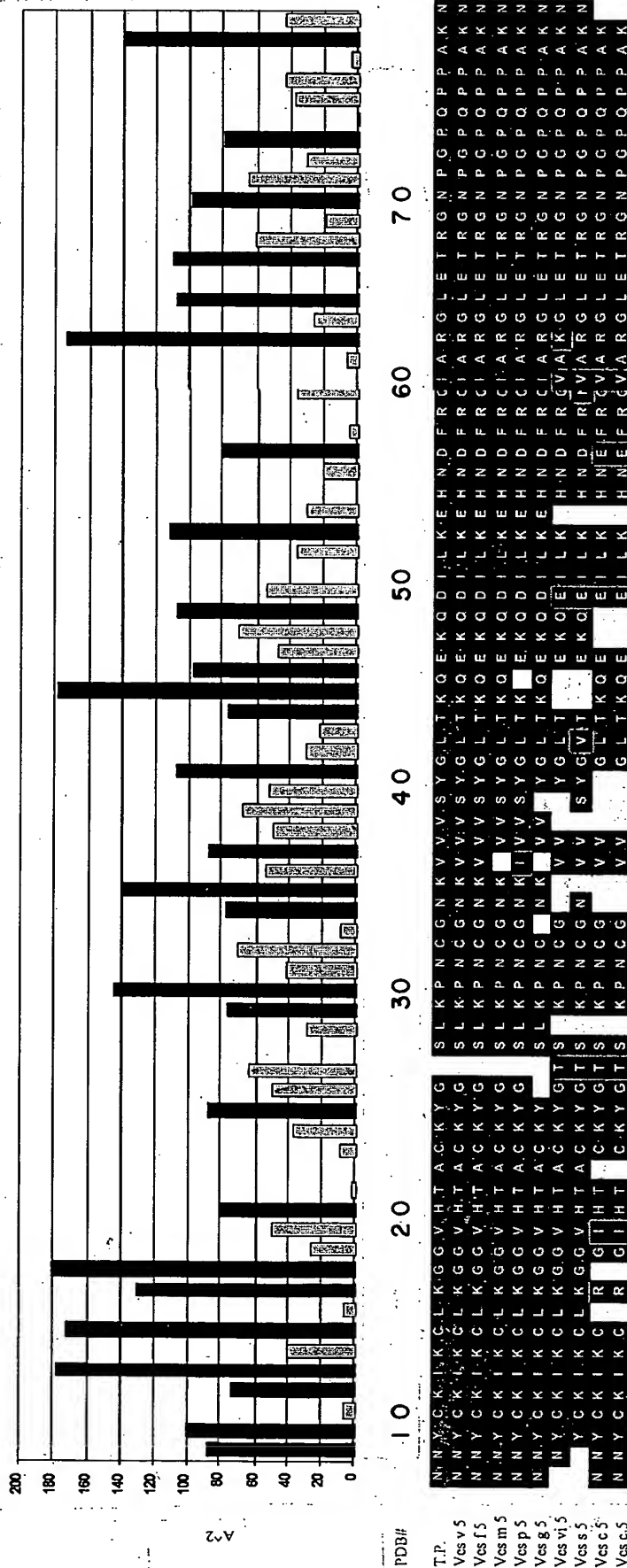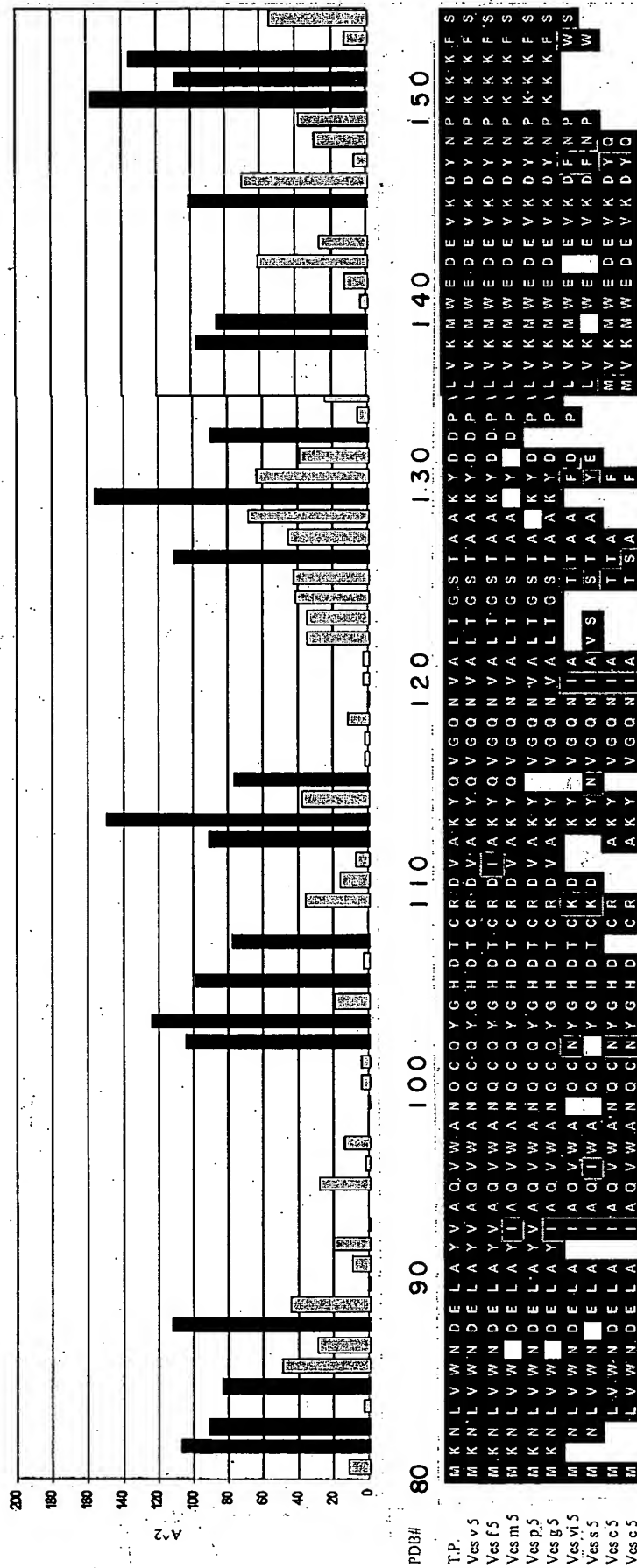
FIG. 11

Mutant-specific oligonucleotide primers used for Ves v 5 mutants.
Mutated nucleotides underlined.

Ves v 5 mutant 1 (K72A)
Ves v 5 sense   5'- ACCACAGCCTCCAGCGAAGAATATGAAAAATTTGGTATGGA -3'
Ves v 5 non-sense   3'- TGGTGTCGGAGGTCGCTTCTTATACTTTTAAACCATACCT -5'
sense primer   5'-   CCAGCGGCTAATATGAAAAAT -3'
non-sense primer   3'-   GTCGGAGGTCGCCGATTATAC -5'

Ves v 5 mutant 2 (Y96A)
Ves v 5 sense   5'- GGCTAATCAATGTCAATATGGTCACGATACTTGCAGGGATG -3'
Ves v 5 non-sense   3'- CCGATTAGTTACAGTTATACCAGTGCTATGAACGTCCCTAC -5'
sense primer   5'-   TGTCAAGCTGGTCACGATACT -3'
non-sense primer   3'-   TTAGTTACAGTTCGACCAGTG -5'

FIG. 12

Oligonucleotide primers for the site directed mutagenesis of Ves v 5.

```
all sense        1 : XhoI start,    38-mer:

                         EcoRI
                 5'- C C G C T C G A G A A A A G A A A C A A T T A T T G T A A A A T A A A A T G
                          L   E   K   R   N   N   Y   C   K   I   K
                     KaX2 cleavage site              amino terminus of Ves v 5


1 sense          1 : K72As      21-mer    5'- C C A G C G G C T A A T A T G A A A A A T
1 non-sense      2 : K72Aa      21-mer    5'- C A T A T T A G C C G C T G G G A G G C T G

2 sense          3 : Y96As      21-mer    5'- T G T C A A G C T G G T C A C G A T A C T
2 non-sense      4 : Y96Aa      21-mer    5'- G T G A C C A G C T T G A C A T T G A T T

all non-sense    7 : CT-pPICZaA, 21-mer   5'- A T T C A T C A G C T G C G A G A T A G G
```

FIG. 13

```
  1 AACAATTATTGTAAAATAAAATGTTTGAAAGGAGGTGTCCATACTGCCTGCAAATATGGA    60
  1 N  N  Y  C  K  I  K  C  L  K  G  G  V  H  T  A  C  K  Y  G     20


 61 AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGTCTAACGAAACAAGAG   120
 21 S  L  K  P  N  C  G  N  K  V  V  V  S  Y  G  L  T  K  Q  E     40


121 AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAAAAAATTGCACGAGGATTGGAG   180
 41 K  Q  D  I  L  K  E  H  N  D  F  R  Q  K  I  A  R  G  L  E     60


                                 1 ( K 7 2A ) ( AAG- GCT )
181 ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAATATGAAAAATTTGGTATGGAAC   240
 61 T  R  G  N  P  G  P  Q  P  P  A  K  N  M  K  N  L  V  W  N     80


                                       2 ( Y9 6A ) ( TA -GC )
241 GACGAGTTAGCTTATGTCGCCCAAGTGTGGGCTAATCAATGTCAATATGGTCACGATACT   300
 81 D  E  L  A  Y  V  A  Q  V  W  A  N  Q  C  Q  Y  G  H  D  T    100


301 TGCAGGGATGTAGCAAAATATCAGGTTGGACAAAACGTAGCCTTAACAGGTAGCACGGCT   360
101 C  R  D  V  A  K  Y  Q  V  G  Q  N  V  A  L  T  G  S  T  A    120


361 GCTAAATACGATGATCCAGTTAAACTAGTTAAAATGTGGGAAGATGAAGTGAAAGATTAT   420
121 A  K  Y  D  D  P  V  K  L  V  K  M  W  E  D  E  V  K  D  Y    140


421 AATCCTAAGAAAAAGTTTTCGGGAAACGACTTTCTGAAAACCGGCCATTACACTCAAATG   480
141 N  P  K  K  K  F  S  G  N  D  F  L  K  T  G  H  Y  T  Q  M    160


481 GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTATAAAATACATTCAAGAGAAATGG   540
161 V  W  A  N  T  K  E  V  G  C  G  S  I  K  Y  I  Q  E  K  W    180


541 CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAAACTTTAAGAATGAGGAACTT   600
181 H  K  H  Y  L  V  C  N  Y  G  P  S  G  N  F  K  N  E  E  L    200


601 TATCAAACAAAGTAA                                                612
201 Y  Q  T  K  stop                                              204
```

FIG. 14

rVes v5

Lys72Ala

Tyr96Ala

% Inhibition

ng inhibitor